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TBEV-complex and *Anaplasma phagocytophilum* in sheep and ticks on the Island of Bornholm in the Baltic Sea

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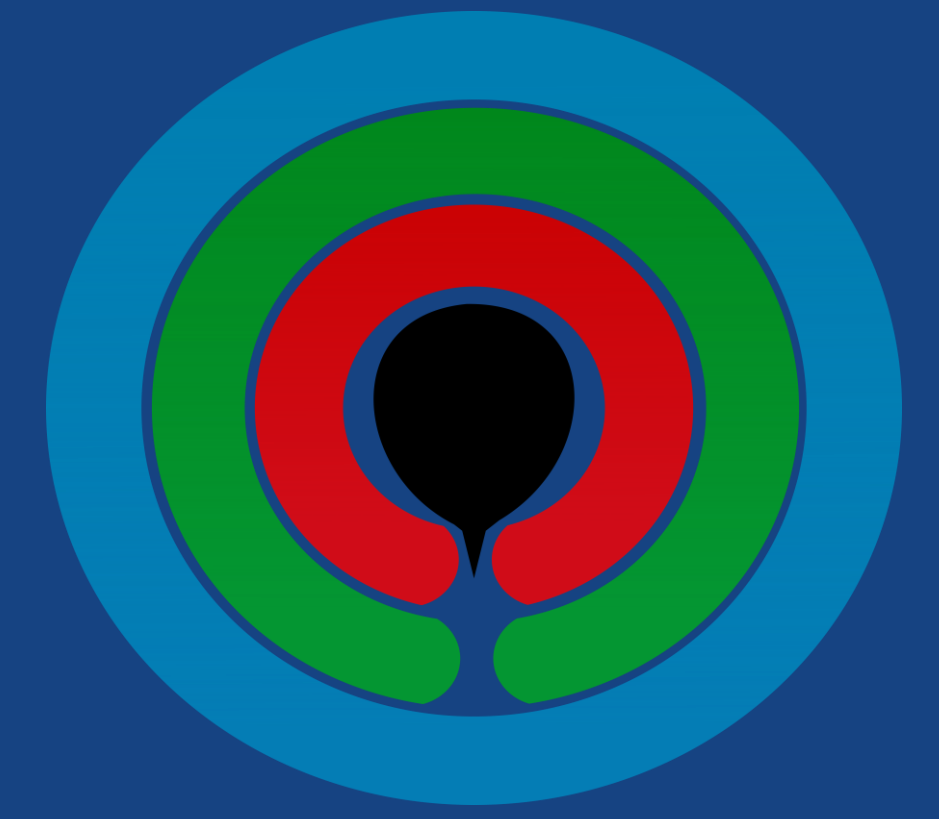
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Objective

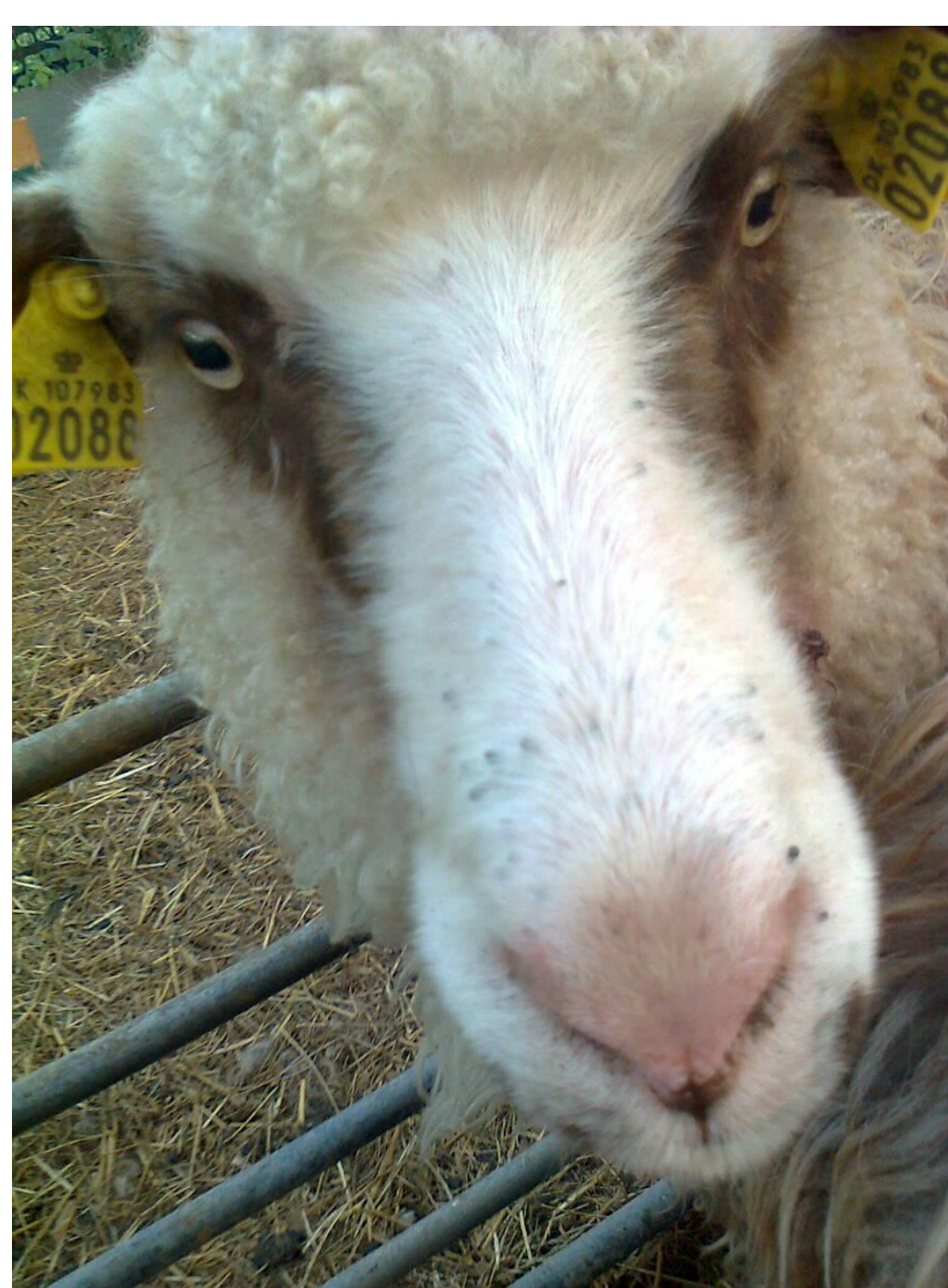
The objective of this study was to determine the seroprevalence of the Tick-borne Encephalitis virus complex and *Anaplasma phagocytophilum* in a sheep flock on the Island of Bornholm in the Baltic Sea and to determine the minimal infectious rate of TBE-virus in ticks collected in the proximity of the grazing areas of the sheep flock.

Introduction

The Tickborne encephalitis virus complex (TBEV-complex) consist of flaviviruses that can cause disease in both animals and humans. In 2004, Jensen et. al. showed that Tickborne encephalitis virus (TBEV) and Louping ill-virus (LIV) is coexisting in ticks on the Island of Bornholm in the Baltic Sea (1). TBEV has been known to result in Tickborne Encephalitis in humans on Bornholm since the 1950s (2), whereas LIV, usually resulting in encephalitis in sheep, never had been described in Denmark (1). Previous studies in sheep propose the need of immunosuppression in order for LIV infection to manifest into encephalitis. This immunosuppression was shown to be caused by simultaneous infection with *Anaplasma phagocytophilum* a bacteria causing TBF (Tickborne Fever) in ruminants and HGA (Human Granulocytic Anaplasmosis) in humans (4,5).

Veterinarian Inga Stamphøj, consulting a sheep farm on the Island of Bornholm, came to us with a specific concern regarding LIV.

A couple of sheep in this farm suffered from symptoms of encephalitis when grazing the Paradise hills (In Danish: Paradisbakkerne). A blood sample from one of these sheep was sent to the Moredun Institute, Scotland where her suspicion was confirmed, the causative agent was a member of the TBEV-complex. For this reason it was recommended that all lambs in the flock should go to this specific pasture soon after birth. Since then no sheep in the flock has suffered from symptoms of encephalitis.



Tick infested sheep.

Even with the lack of new cases of encephalitis she was still concerned that the ticks in this pasture were harbouring LIV/TBE with the potential to cause sickness in both sheep and humans. We decided to investigate this further.



The recreational area of Paradise Hills where the sheep flock grazes, Bornholm, Denmark.

Method

41 sheep grazing the Paradise hills were blood sampled in late June 2014. The sheep was all older than 1 year, female and considered healthy upon examination. The samples were tested for the presence of *A. phagocytophilum* antibodies using a modified commercial indirect immunofluorescence assay test – IFA (Focus Diagnostics, California, USA), replacing the conjugate with diluted (1:10) FITC-labelled antibody to sheep IgG (H+L) produced in rabbit (SeraCare, KPL Antibodies and Reagents, Gaithersburg, USA) and hereafter sent to the Department of Virology, Medical University Vienna where they were examined for the existence of TBEV-complex specific antibodies by virus neutralisation test (VNT). 247 ticks collected by flagging in the proximity of the Paradise hills in late July 2014 were tested for the presence of TBEV in pools of 8 to 11 individuals (29 pools) using real time-RT-PCR (Schwaiger & Cassinotti, 2003) (6).

Results

All 41 sheep samples had antibodies against *A. phagocytophilum* giving a prevalence of 100%. 23 of 41 samples was VNT-positive, 11 negative and 7 samples was not able to be determined due to toxic effect of the serum on the cell, this yielding a seroprevalence of the TBE-virus complex of 68% in the sheep (n=34), Figure 1. No tick pools were PCR positive.

Discussion

Giving the results it is safe to say that members of the TBE-virus complex must still be circulating in the ticks at the Paradise hills on the Island of Bornholm. Due to the possible coexistence of LIV and TBEV on the Island of Bornholm it is difficult to say which of the viruses is causing the seroconversion in the sheep.

We examined 247 ticks in the area around Paradise hills and in spite of this we found no TBE-positive ticks, once again showing that ticks is a bad indicator of diseases with such low prevalence.



Ixodes ricinus
Questing female.

It is important to characterise the circulating virus. If a sheep shows signs of encephalitis it would be interesting to characterise and isolate the virus from the brain of the sheep. Another possibility could be the trapping and examination of small rodents since they maintain the virus for a longer period of time. The high presence of *A. Phagocytophilum* antibodies suggest a potential presence of immunosuppression and hence an ability of LIV to cause encephalitis in the sheep in this specific area, if the virus is circulating.

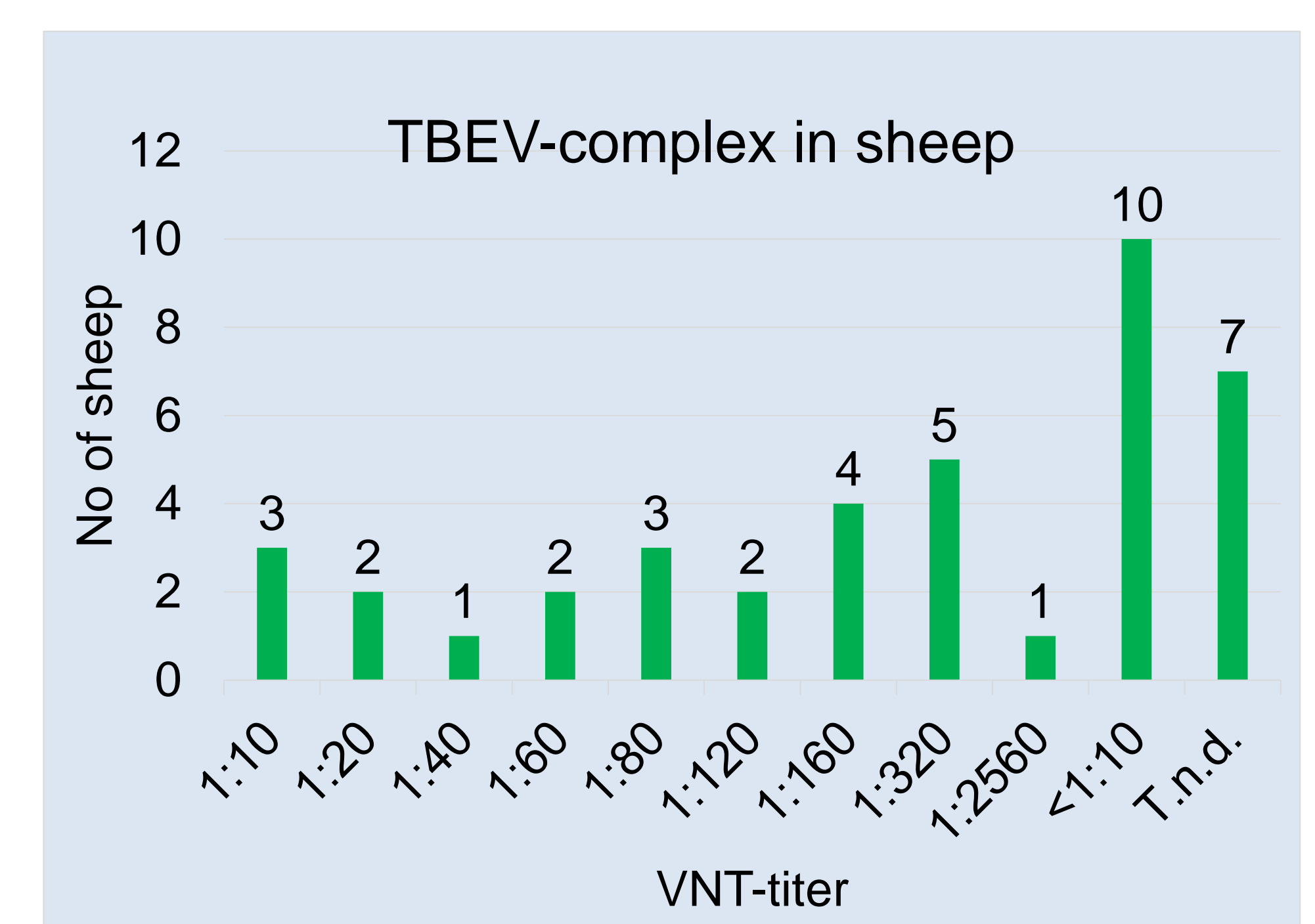


Figure 1: Displays the virus neutralisation test (VNT) titers in sheep grazing the Paradis hills on Bornholm. Titers below 1:10 were considered negative. T.n.d. = toxic effect of the serum on the cell

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Note: Since the submission of the abstract the results of the RT-PCR on ticks from Paradise hills has been added. For this reason the title has been changed and authors Bestehorn M, Chitimia-Dobler L and Dobler G is added due to their aiding in the conduction of the RT-PCR of the ticks and the completion of this poster.

